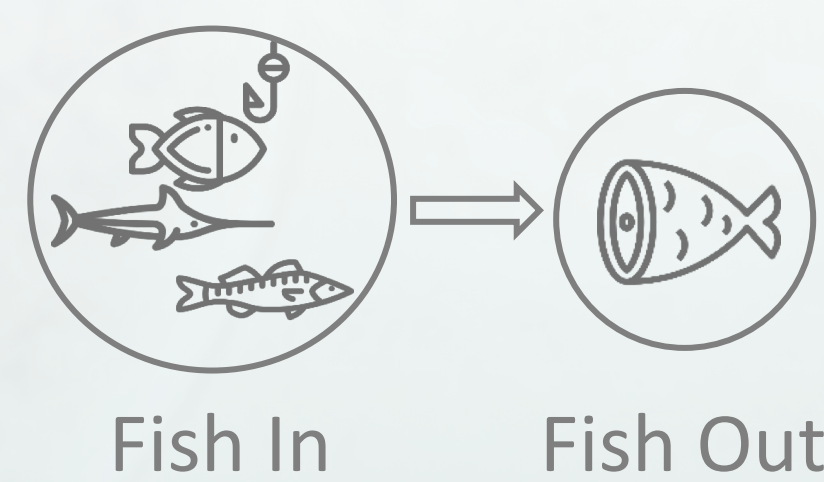


INTRODUCTION

Fisheries and aquaculture are a major source of employment and income on which the livelihoods of 10-12% of the world's population are based. However, the production process has to be improved in order to increase the sustainability. The ratio of Kg of wild fish needed to produce one kilogram of fish called Fish In Fish Out (FIFO), the carbon and water footprint and the discharge of nitrogen and phosphorus into the environment are certain points that must be resolved to increase the sustainability of this industry.

Globally, the **FIFO value** of marine fish has been declining to 0.53 in 2015, which means that for every kilogram of wild fish, 1.9 kilograms of farmed fish is produced [1]. This decrease is due to an increase in alternative sources of fishmeal and fish oil such as oleaginous seeds, rich in Polyunsaturated fatty acids (PUFAs).



Microalgae and aquaculture have traditionally been grown in both freshwater and seawater, two activities that have always been linked due to microalgae are part of the food chain as primary producers and feed different species in larval stages. They are now playing an increasingly important role in the world of wastewater treatment [2] but in the field of aquaculture, this technology is less technologically advanced and often linked to extensive systems [3]. Since the main problem of alternative sources of proteins and fatty acids of terrestrial origin do not have adequate profiles, some authors [4,5] propose the use of microalgae as a potential substitute for fishmeal and fish oil.

OBJECTIVES

1. To study the feasibility of applying **microalgae technologies** to effluents from intensive aquaculture in order to recycle nutrients.
2. To study the feasibility of **incorporation the microalgae** biomass obtained in the treatment process as a **percentage of fish feed**.

MATERIAL & METHODS

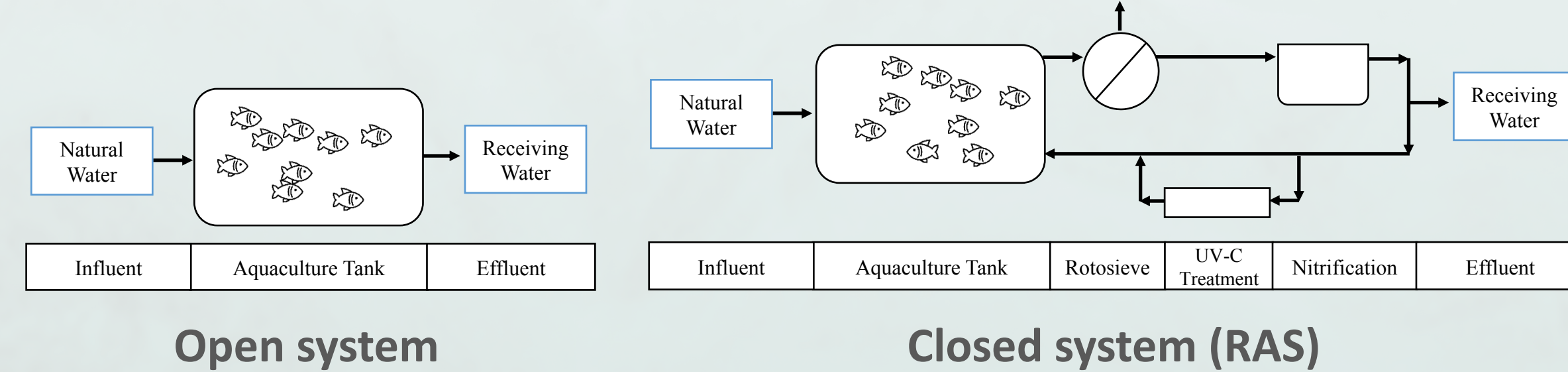
Phase I

Bibliographic review of microalgae species with rapid growth and consumption of nutrients, and whose biochemical composition is interesting to incorporate directly into fish feeds. The selected microalgae were:

1. *Nannochloropsis gaditana*
2. *Pavlova lutheri*
3. *Isochrysis galbana*
4. *Tetraselmis chuii*
5. *Phaeodactylum tricornutum*
6. *Chaetoceros gracilis*
7. *Bloom* from aquaculture effluent

Phase II

Characterization of 4 aquaculture effluents from two systems that are currently applying in intensive production aquaculture industry: **open and closed** (or Recirculation Aquaculture System, RAS) **systems**. The 4 characterized effluents are: open system effluent, elutriate from biosolids aerobic digestion, RAS effluent with partial nitrification and RAS effluent with total nitrification.



Phase III

Studies of **growth kinetics** in batch and semi-continuous, **nutrient consumption**, **biomass productivity** and **quality of the 6 species selected and the bloom** using the effluent selected in phase II.



The experiments were conducted following 5 steps, the first 4 under laboratory conditions (22°C, 24h of light) and, the last one, under environmental conditions.

Experimental Set Up

Step 1: micronutritional requirements

(Volume= 1L, 4 reactors per microalgae)



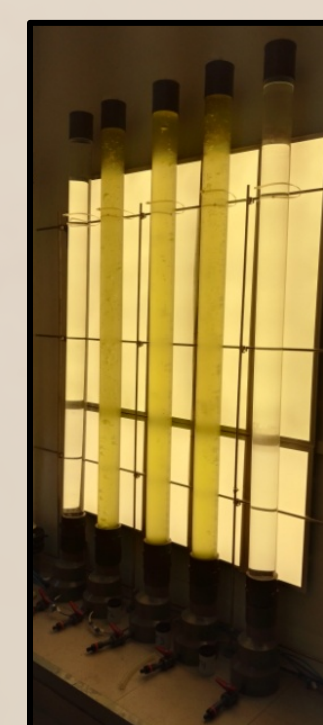
Step 2: pre-scaling

(Volume = 8L, 4 reactors per microalgae)



Step 3:

Batch operation
(Volume = 18L, 3 reactors per microalgae)



Step 4:

Semi-continuous operation
(Volume = 18L, 1 reactor per microalgae)



Step 5:

Batch at pilot scale
(Volume = 450L, 1 raceway per microalgae)



RESULTS

Effluents characterization

Effluent	TDP (mg L ⁻¹)	TDN (mg L ⁻¹)	N-NO ₂ (%)	N-NO ₃ (%)	N-NH ₄ (%)
Open System	0.05	1.1	34	15	54
Elutriate	2.5	22	88	11	1
RAS	0.24	10	100	0	0
(Complete nitrification)					
RAS	0.24	10	34	15	54
(Incomplete nitrification)					

Modeling

Batch growth kinetics

Verhulst logistic kinetic model

Productivity [6]

$$\frac{\delta X(t)}{\delta t} = \mu X(t) \left[1 - \frac{X(t)}{X_{max}} \right] \quad Productivity = \frac{\mu (0.9 X_{max} - 1.1 X_0)}{\ln \left(\frac{9 (X_{max} - 1.1 X_0)}{1.1 X_0} \right)}$$

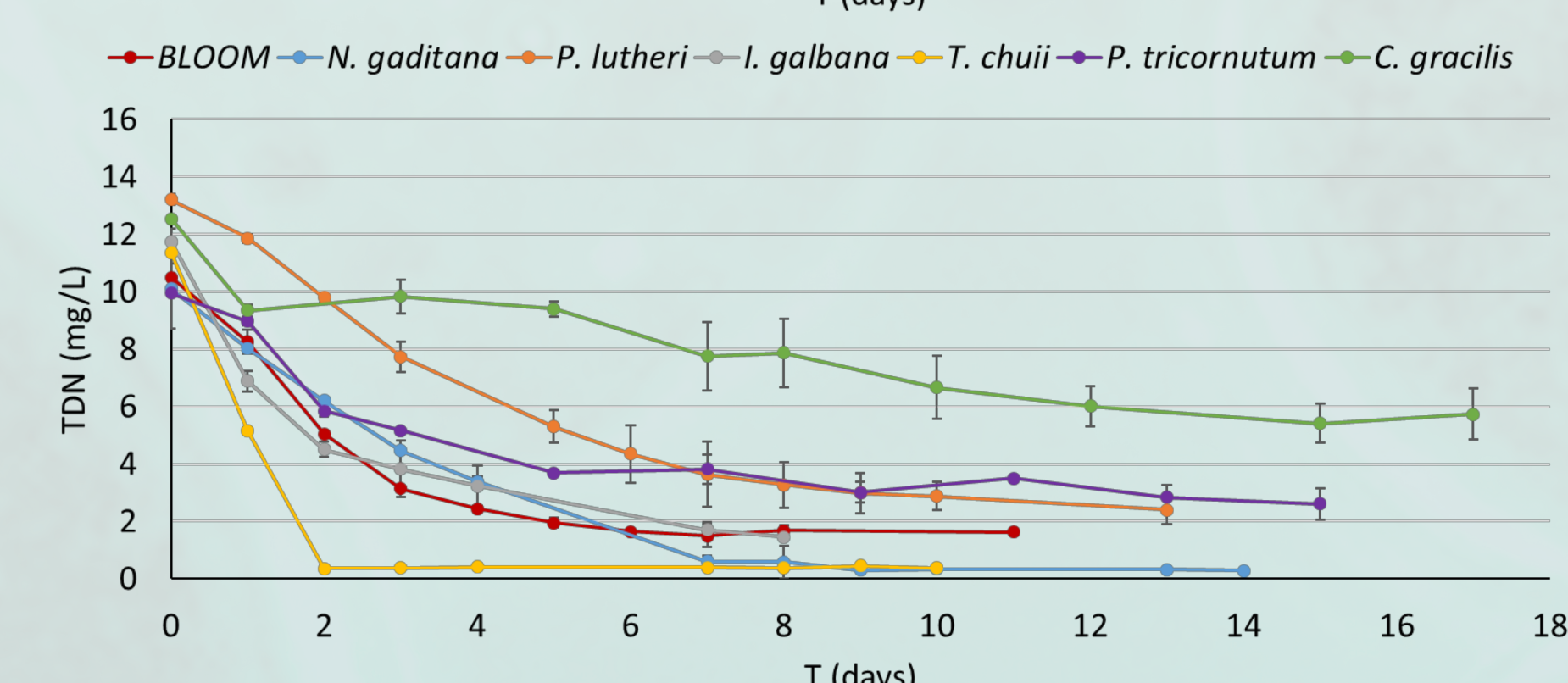
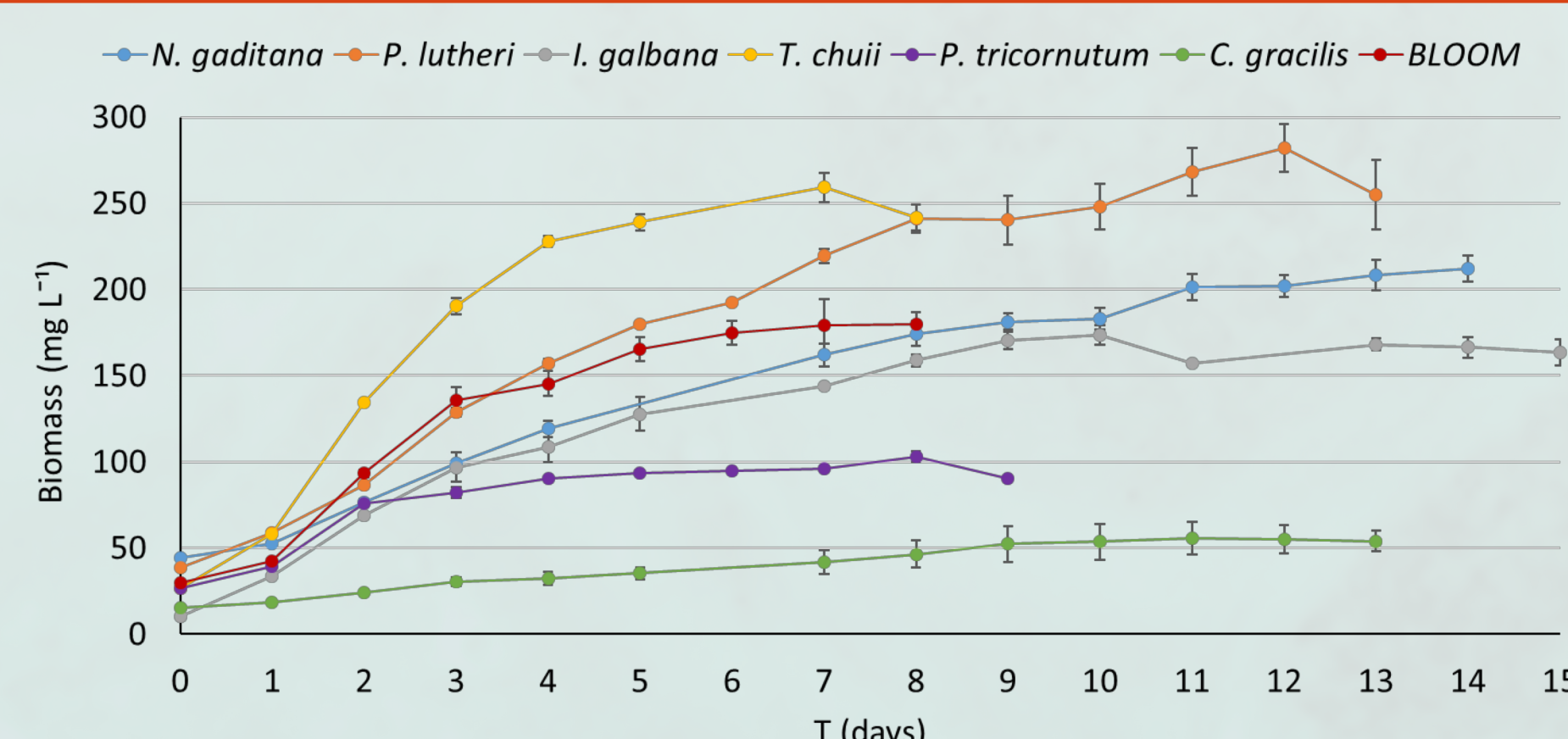
Nutrient removal kinetics

$$S = \frac{\left(\frac{X_0}{Y_0} + S_0 \right) \cdot (S_0 - S_{na}) - S_{na} \left(S_0 - \left(\frac{X_0}{Y_0} + S_0 \right) \right) \cdot e^{\mu t}}{(S_0 - S_{na}) - \left(S_0 - \left(\frac{X_0}{Y_0} + S_0 \right) \right) \cdot e^{\mu t}}$$

The experiments were conducted by using the concentration and speciation of the nutrients corresponding to a RAS with incomplete nitrification. Once selected the nutrients concentration, micronutritional (Metals and Vitamins) requirements essays were done. Results indicates that each microalgae has different micronutritional requirements to grow up properly. To obtain enough inoculum for 18 L reactors, 28L were culture just adding 10 mg L⁻¹ of TDN and 0.2 mg L⁻¹ of TDP.

The six microalgae and the bloom was tested in batch by triplicate and in semi-continuous operation using one reactor. The higher concentration (X_{max}) in batch was obtained with *T. chuii* and *P. lutheri*. Lower Hydraulic Retention Time (HRT) was reached using *P. tricornutum* and *T. chuii*. Finally, the two microalgae with higher productivity calculated in batch operation was the bloom and *T. chuii*.

Total Dissolved Phosphorous (TDP) was 100% removed in every case in less than 24h. For Total Dissolved Nitrogen (TDN), *T. chuii* was the specie that removed TDN faster, more than 99% in less than 2 days.



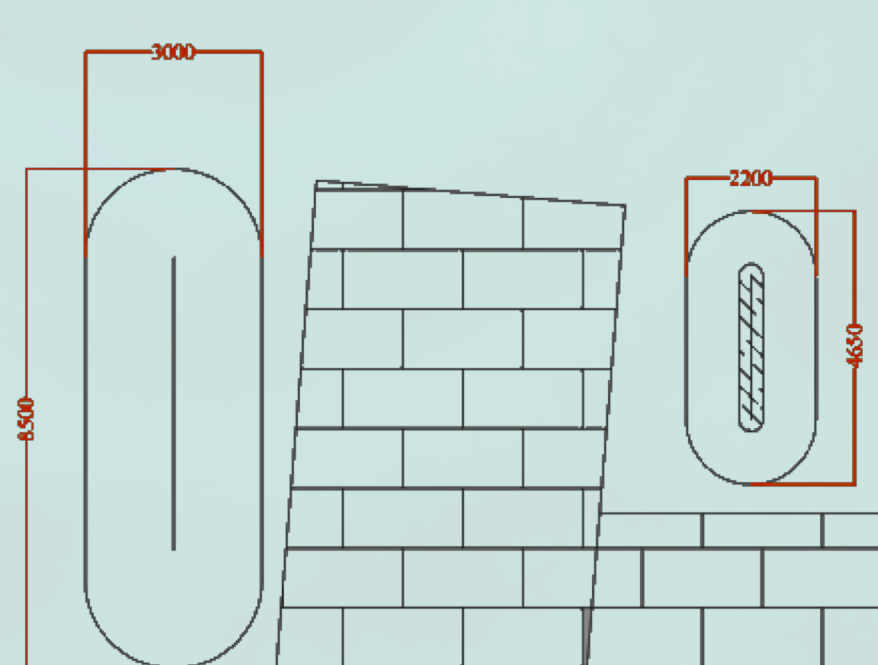
CONCLUSIONS

1. Each microalgae have different micronutritional requirements, that must to be taken into account.
2. *T. chuii*, *P. tricornutum* and the Bloom were the microalgae with better performance under batch operation.
3. The semi-continuous operation generates the necessary information to know the behavior of the microalgae in the long term.
4. The study of all the variables that have been taken into account in these experiments such as production, maximum concentration, hydraulic retention time or PUFAs content will determine which is the best microalgae to scale the test using real RAS effluent.

REFERENCES

- [1] IFFO (2017). Fish In: Fish Out (FIFO) ratios for the conversion of wild feed to farmed fish, including salmon. Retrieved September, 2018 from <http://www.ifo.net/fish-fish-out-fifo-ratios-conversion-wild-feed>
- [2] Arbib, Z. et al., (2014). Capability of different microalgae species for phytoremediation processes: wastewater tertiary treatment, CO₂ bio-fixation and low cost biofuels production. *Wat. Res.*, 49, 465-474
- [3] Milhazes-Cunha, H., & Otero, A. (2017). Valorisation of aquaculture effluents with microalgae: The Integrated Multi-Trophic Aquaculture concept. *Algal Res.*, 24, 416 – 424
- [4] Perez-Velázquez (2018). Partial replacement of fishmeal and fish oil by algal meals in diets of red drum *Sciaenops ocellatus*. *Aquaculture*, 487, 41-50
- [5] Madeira et al., (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest. Sci.*, 205, 111-121
- [6] Ruiz et al., (2013). Photobiotreatment model (PhBT): a kinetic model for microalgae biomass growth and nutrient removal in wastewater. *Environ. Technol.* 34 (5 – 8), 979 – 991

NEXT STEPS



Pilot Scale Operation

Once laboratory and environmental scale experiments are conducted, a raceway at **pilot scale** (6 m³) is being built in a real aquaculture facility. One year of monitoring is going to be carried out to optimize the operation of this reactor.



Fish Feed Essays

The microalgae biomass produced in the raceway reactors at pilot scale will be tested as an additive in fish feed. This test will be performed in order to reduce fishmeal and/or fish oil percentage in fish feed and, therefore, the **FIFO rate**.

ACKNOWLEDGEMENTS

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